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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	R. Chalifour et al.	Art Unit:	1647
Serial No.:	09/724,842	Examiner:	S. Turner
Filed:	November 28, 2000	Customer No.:	21559
Title:	Vaccine for the Prevention and Treatment of Alzheimer's and Amyloid Related Diseases		

Commissioner for Patents
P.O. Box 1450
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DECLARATION OF FRANCINE GERVAIS, PH.D.

Considered 9-23-03

I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application.

2. I hold the position of Vice President, Research and Development, for the assignee of record of the application, Neurochem, Inc.

3. The experiments described below were carried out by me or under my supervision

and, as will be described further below, show that D-peptides provide significant advantages when compared to L-peptides in vaccination against β-amyloid peptides.

4. The graphs in Figures 1-4 (attached) show the antibody responses of rabbits immunized with L and D versions of immunogenic fragments of amyloid β (Aβ) peptides. Figures 1 and 2 show antibody titers in rabbits immunized with D- and L-peptides including amino acids 10-22 of Aβ. Figure 1 shows that a rabbit (#3216) immunized with BSA-(C)D10-22 had substantially higher antibody titers than a rabbit immunized with a corresponding L-peptide (BSA-(C)L10-22) (nearly 25,000 vs. undetectable). Similarly, Figure 2 shows that a rabbit (#3224) immunized with another D-peptide construct, KLH-(C)D10-22 had substantially higher antibody titers than a rabbit immunized with the corresponding L-peptide (KLH-(C)L10-22) (nearly 25,000 vs. undetectable). Figures 3 and 4 show antibody titers obtained in rabbits immunized with D- and L-peptides including amino acids 13-22 of Aβ. Figure 3 shows that a rabbit (#3213) immunized with BSA-(C)D13-22 had higher antibody titers than a rabbit immunized with a corresponding L-peptide (BSA-(C)L13-22) (approximately 300 vs. undetectable), while Figure 4 shows similar results for another rabbit (#3214) (approximately 900 vs. undetectable). It is to note that in Figure 3 the rabbit immunized with D13-22 has not yet reached its plateau for antibody production. The antibody titer is expected to reach much higher levels with more time. These experiments show that the D version of the peptides tested induce higher antibody titers in rabbits than the corresponding L peptides.

5. The following experiment was carried out in TgCRND8 transgenic mice, which

express the human amyloid precursor protein (APP) gene and develop Alzheimer's-like pathology. Figure 5 is a table showing the levels of Aβ40 and Aβ42 in soluble and insoluble fractions of brains from TgCRND8 mice that had been immunized with D13-21-KLH, L13-21-KLH, or KLH. Figure 6 is a table providing comparisons of the results that are shown in the table of Figure 5. In particular, Figure 6 shows that immunization with D13-21-KLH led to decreases in the levels of Aβ40 in soluble (26%) and insoluble (13%) fractions, and decreased levels of Aβ42 in the insoluble fraction (19%), when compared to immunization with KLH alone. In contrast, immunization with L13-21-KLH led to increased levels of Aβ40 in soluble (10%) and insoluble (32%) fractions and increased levels of Aβ42 in the soluble fraction (64%), as compared to KLH alone. The overall decrease in Aβ40 and Aβ42 levels in both soluble and insoluble fractions was greater with the D-peptide, as compared with the L-peptide (33%, 34%, 21%, and 15% in the Aβ40 soluble, Aβ40 insoluble, Aβ42 soluble, and Aβ 42 insoluble fractions, respectively). Decreased levels of Aβ peptides in the brain is a desirable effect of this treatment, as increased levels of Aβ peptides in the brain is associated with Alzheimer's disease.

6. In the following experiments, brain Aβ levels were also measured in TgCRND8 mice immunized with D13-21-KLH, L13-21-KLH, or KLH. In addition, Aβ levels in plasma were also determined. Figure 7 is a table showing the levels of Aβ40 and Aβ42 in soluble and insoluble fractions of brains from TgCRND8 mice immunized with D13-21-KLH, L13-21-KLH, or KLH. Figure 8 is a table providing comparisons of the results that are shown in the table of Figure 7. These comparisons show that immunization with the D-peptide led to decreased levels

of Aβ40 in the soluble fraction (5%) and decreased levels of Aβ42 in both soluble (4%) and insoluble (13%) fractions, as compared to the levels in KLH-treated mice. In contrast, levels of Aβ40 in the insoluble fraction (72%) and levels of Aβ42 in both the soluble (27%) and insoluble (6%) fractions increased in L13-21-KLH-immunized mice, as compared to KLH controls. The overall decrease in Aβ40 insoluble and Aβ42 soluble and insoluble fractions was greater with the D-peptide, as compared with the L-peptide (32%, 24%, and 18% in Aβ40 insoluble, Aβ42 soluble, and Aβ insoluble, respectively).

Figure 9 is a table showing the levels of Aβ40 and Aβ42 in the plasma of TgCRND8 mice immunized as described above, and Figure 10 is a table showing comparisons of the data set forth in Figure 9. These data show that immunization with both D and L peptides led to an increase in plasma levels of both Aβ40 (58% for D and 28% for L) and Aβ42 (47% for D and 24% for L), when compared to KLH. It is striking to see that the increase in Aβ plasma levels, both for Aβ40 (23%) and Aβ42 (19%), is greater for D peptides, as compared to L peptides (showing about a two-fold increase of Aβ40/42 plasma levels). Increased levels of Aβ peptides in the plasma is a desirable effect of this treatment, as it may mean that there are in turn decreased levels of these peptides in the brain, where accumulation of the peptides is associated with Alzheimer's disease. The effect of D peptides at raising plasma Aβ levels was surprising, as previous studies using L peptides had not reported a change in these levels.

7. In additional experiments, TgCRND8 transgenic mice were immunized with Aβ L- and D-peptides as follows. Mice immunized with KLH-Cys-L10-15-D16-21 showed a 42%

reduction in the levels of soluble Aβ42 (pg/g wet brain) in the brain, as compared to a control, whereas mice immunized with the all-L peptide showed no reduction in soluble Aβ42 levels. In addition, as seen in TgCRND8 mice immunized with KLH-cys-D13-21, which showed significantly higher antibody titers than those seen in mice immunized with KLH-cys-L13-21 (10,933 vs. 84), the same was found for immunization with KLH-cys-L10-15-D16-21 (8682) vs. KLH-cys-L10-21 (100).

8. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: July 2nd 2003

Francine Gervais

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